polymerase, wherein at least one of said at least two thermostable DNA polymerases has reverse transcriptase activity;

wherein the at least two DNA polymers are mixed so that conversion of the RNA to the DNA will be conducted in the presence of the at least two thermostable DNA polymerases.

F2 Cont

REMARKS

Claims 1-126, 132-137 and 141-145 are pending in this application. By this Amendment, claims 1 and 37 are amended. No new matter is added.

The Office Action rejects claims 1-126, 134-137 and 143-146 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 34-58 of copending Application No. 09/257,166 in view of Gelfand et al. (U.S. Patent Application No. 5,310,652) and Birch et al. (U.S. Patent Application No. 5,677,152). The Office Action also rejects claims 1-126, 134-137 and 143-145 under 35 U.S.C. 103(a) as being obvious over the combination of Köster et al. (U.S. Patent No. 5,928,906) in view of Gelfand et al. and Birch et al. Similarly, claims 132-133 and 141-142 are rejected under 35 U.S.C. 103(a) as being obvious over the combination of Köster et al. in view of Gelfand et al. and Birch et al. and further in view of Hill (U.S. Patent No. 5,525,492). These rejections are traversed.

As the Office Action notes, Köster et al. disclose methods "requiring two different polymerases..." As the Office Action also notes, the Köster et al. patent does not disclose DNA polymerase-mediated reverse transcription coupled to PCR amplification. However, the Office Action asserts that one of ordinary skill in the art would have been

motivated to modify the method of Köster et al. by application towards RNA using a polymerase with reverse transcriptase activity because Gelfand et al. disclosed the advantages of combined reverse-transcription and amplification.

However, Köster et al. already discloses that the nucleic acid molecule in their process can be "RNA if combined with reverse transcription to generate a DNA. For example, reverse transcription can be performed using a suitable reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) using standard techniques..." (col. 6, lines 23-35).

None of the cited references teach or suggest using a first DNA polymerase to amplify DNA to produce truncated copies of a DNA and a second DNA polymerase to generate full length copies of the DNA, as required by the present claims.

There would have been no reason to modify Köster et al. towards RNA since Köster et al. already contemplates a combined transcribing, amplifying and sequencing method in which a reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) is used to generate a DNA and then two different polymerase enzymes, each having a different affinity for the particular chain terminating nucleotide, are used.

Applicants respectfully note that Köster et al. nowhere teaches or suggests that the reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) also be used as one of two polymerase enzymes for sequencing.

Gelfand et al. fails to make up for deficiencies in Köster et al. since Gelfand et al. also fails to suggest a reverse transcriptase used for transcribing (e.g., Moloney leukemia virus reverse transcriptase) also be used as one of two polymerase enzymes for sequencing.

Additionally, there would have been no motivation to replace one of the two Köster et al. polymerase enzymes with a polymerase of Gelfand et al. since any Köster et al. RNA would have already been converted to CDNA using the "suitable reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) using standard techniques."

Furthermore, Applicants respectfully note that none of the applied references teach or suggest conducting transcription in the presence of at least two thermostable DNA polymerases. Thus, in order to expedite prosecution of this application and to make even more clear the patentability of the present invention over the applied combinations of references (and in overcoming the double patenting rejection), the present claims have been amended to even more clearly require that "the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable DNA polymerases." Applicants do not believe that any proper motivation has been or could be shown to modify any of the teachings of the cited references to reverse transcribe in the presence of two thermostable DNA polymerases.

Additionally, regarding claims requiring "at least one polymerase-inhibiting agent," Applicants respectfully note that none of the applied references teach or suggest such a "hot-start" agent in a sequencing reaction with at least the thermostable polymerases, as required in the present claims 64-99 and 122-144.

For at least the above reasons, reconsideration and withdrawal of the rejections under the judicially created doctrine of obvious-type double patenting and under 35 U.S.C. § 103(a) are respectfully requested.

In view of the above amendments and remarks, Applicants respectfully submit that this application is in condition for allowance. Favorable consideration and prompt allowance of the claims is earnestly solicited. Should the Examiner believe anything further is desirable in order to place this application in even better condition for allowance, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300, making reference to Attorney Docket No. 101614-08090.

Respectfully submitted,

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MARKED-UP COPY OF AMENDED CLAIMS

1. (Amended) A method for sequencing at least a portion of a RNA involving converting the RNA to a DNA and simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of

(a) subjecting a miexture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises

said RNA,

a buffer solution,

a first primer which is able to hybridize with a strand of said DNA,

a second primer which is able to hybridize with a strand of said DNA

complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labeled,

deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide

derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP,

at least one dideoxynucleotide or another terminating nucleotide, and at least two thermostable DNA polymerases, wherein said at least two

thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, wherein one of said at least two thermostable DNA polymerases has reverse transcriptase activity,

to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers;

(b) separating at least said truncated copies to make a sequence ladder; and thereafter

(c) reading the sequence ladder to obtain the sequence of said at least a portion of said RNA wherein the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable DNA polymerases.

37. (Amended) A kit for sequencing at least a portion of a RNA, comprising

deoxynucleotides or deoxynucleotide derivatives, which deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP;

at least one dideoxynucleotide or another terminating nucleotide; and

at least two thermostable DNA polymerases, wherein said at least two

thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide in comparison to said first thermostable DNA polymerase, wherein at least one of said at least two thermostable DNA polymerases has reverse transcriptase activity;

wherein the at least two thermostable DNA polymerases are mixed so that conversion of the RNA to the DNA will be conducted in the presence of the at least two thermostable DNA polymerases.